Knock-In Mouse Models of Huntington's Disease

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Summary: Huntington's disease is an autosomal dominant neurodegenerative disorder that is characterized by motor, cognitive, and psychiatric alterations. The mutation responsible for this fatal disease is an abnormally expanded and unstable CAG repeat within the coding region of the gene encoding huntingtin. Numerous mouse models have been generated that constitute invaluable tools to examine the pathogenesis of the disease and to develop and evaluate novel therapies. Among those models, knock-in mice provide a genetically precise reproduction of the human condition. The slow progression and early development of behavioral, pathological, cellular, and molecular abnormalities in knock-in mice make these animals valuable to understand the early pathological events triggered by the mutation. This review describes the different knock-in models generated, the insight gained from them, and their value in the development and testing of prospective treatments of the disease. **Key Words:** Knock-in, mouse models, Huntington's disease, behavior, huntingtin, aggregates.

INTRODUCTION

Huntington's disease (HD) is a dominantly inherited disorder characterized by a progressive neurodegeneration of the striatum that also involves other regions, primarily the cerebral cortex.[1] Patients display progressive motor, cognitive, and psychiatric impairment.[2] Symptoms usually start at midlife. The mutation responsible for this fatal disease is an abnormally expanded and unstable CAG repeat within the coding region of the gene encoding huntingtin.[3] The pathogenic mechanisms by which mutant huntingtin cause neuronal dysfunction and cell death remain uncertain.

Because the disease is caused by a single mutation, several mouse models[4] have been generated since the isolation of the mutation in 1993.[3] These mouse models are providing insight into the disease pathogenesis and are invaluable tools for the evaluation of potential therapeutic approaches. Three types of mouse models have been developed: knockout, transgenic, and knock-in models. Huntingtin knockout models were the first models generated.[5–8] Although these models are not good models of the disease because nullizygous animals die during embryonic development, they have demonstrated that huntingtin plays a crucial role in embryogenesis. Furthermore, the neuronal degeneration and behavioral phenotype detected in conditional knockout, in which the huntingtin gene was inactivated in brain and testis at early stages, indicate that huntingtin is required for neuronal function and survival.[8a] Transgenic models are those in which the human mutant huntingtin (*HD*) gene, or a fragment of it, is inserted randomly into the mouse genome. In this case, the mouse will express a full-length or a fragment of the mutant gene in addition to the two normal copies of the endogenous mouse huntingtin (*Hdh*) gene. Knock-in mouse models have the mutation inserted into the mouse huntingtin gene and can be homozygous or heterozygous for the mutation. Because knock-in mice carry the mutation in its appropriate genomic and protein context, they are the most faithful genetic models of the human condition. The behavioral, cellular, molecular, and pathological features of knock-in mouse models of HD are presented in this review.

BEHAVIORAL ABNORMALITIES

Initial results with knock-in models (Table 1) were perceived as disappointing because the first abnormal behavioral phenotype described was an increase in aggression in knock-in mice with 72–80 CAG repeats from 3 months of age.[9] Knock-in mice didn't display the overt motor deficits observed in the first, and most studied, transgenic mouse model, namely R6/2 mice.[10,11] However, a closer examination of other knock-in models, with longer CAG repeats, revealed that early subtle be-

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TABLE 1. *Knock-In Mouse Models of HD*

Line Name	Original Strain*	CAG Number and Genetic Insertion	Behavioral Phenotype	Cellular and Molecular Phenotype	Neuropathology†	References
HdhQ50	129Sv × CD1	Chimeric *HD/Hdh* exon 1 with 48 CAGs	No (up to 6 m)	14 m: increased Rrs1 mRNA	No abnormalities (until 6 m)	8, 26, 61
HdhQ92	129Sv × CD1	Chimeric *HD/Hdh* exon 1 with 90 CAGs	No (up to 17 m)	8.5 m: increased Rrs1 mRNA, 9 m: somatic instability.	5 m: nuclear staining, 12 m: nuclear microaggregates and inclusions	20, 26, 61
HdhQ111	129Sv × CD1	Chimeric *HD/Hdh* exon 1 with 109 CAGs	24 m: gait deficits	3 w: increased Rrs1 mRNA, 1.5–2.5 m: decreased cAMP levels, 5 m: decreased BDNF and phospho-CREB levels, somatic instability	1.5–2.5 m: nuclear staining, 4.5–5 m: nuclear microaggregates, 12 m: intranuclear inclusions consistently, 17: neuropil aggregates, 24 m: gliosis	17, 20, 26, 48, 61, 67
Hdh71	129Sv × C57BL/6J	Chimeric *HD/Hdh* exon 1 with 71 CAGs	No (up to 1 y)	Not observed.	No aggreagetes (up to 22 m)	14, 19, 60
Hdh94	129Sv × C57BL/6J	Chimeric *HD/Hdh* exon 1 with 94 CAGs	2 m: hyperactivity, 4 m: hypoactivity, 24 m: Gait deficits	~3 m: increased sensitivity to NMDA, 4 m: decreased enkephalin mRNA levels	6 m: nuclear staining and microaggregates, consistently. 18 m: intranuclear inclusions	14, 19, 60
Hdh140	129Sv × C57BL/6J	Chimeric *HD/Hdh* exon 1 with 140 CAGs	1 m: hyperactivity, 4 m: hypoactivity, 12 m: gait deficits	n.r.	4 m: nuclear staining and aggregates and neuropil aggregates, consistently	13
Hdh6/72 Hdh 4/80	Sv129 × C57BL/6J, Sv129 × FVB/N	72 and 80 CAGs, respectively	~3 m: aggression, 4 m: rotarod deficits‡	9 m: somatic instability	4 m: nuclear staining, 11 m: nuclear aggregates, >11 m: neuropil aggregates, 17–22 m: axonal degeneration 21–27 m: intranuclear inclusions	9, 27, 35, 46, 47, 49
Hdh(CAG)80	129/Ola × C57BL/6J	80 CAGs	>10 m: gait deficits,	n.r.	Nuclear inclusion rarely observed (up to 22 m)	18
Hdh(CAG)150	129/Ola × C57BL/6J	150 CAGs	4–10 m: gait and rotarod deficits, clasping, hypoactivity	4 m: somatic instability, 11 m: clapain levels increased	7–8 m: nuclear staining 14 m: gliosis, nuclear aggregates and inclusion, neuropil aggregates axonal degeneration, degenerated cytoplasmic organels	18, 34, 45, 47

HD = human huntingtin gene; *Hdh* = mouse huntingtin gene; m = months of age; w = weeks of age; y = year old; n.r. = not reported; CREB, cAMP-responsive element binding protein.

*Numerous lines have been backcrossed to various genetic backgrounds.

†No cell loss was detected in knock-in models.

‡Examinated in Hdh6/72 knock-in mice only.

havioral abnormalities were present at an early age.[12–14] Knock-in mice with 94 CAG repeats displayed a biphasic motor behavior, characterized by increased motor activity at 2 months of age, followed by hypoactivity at 4 months[14] In animals with 140 CAG repeats, a similar pattern of motor abnormalities was observed.[13] Furthermore, in agreement with the relationship between CAG repeat length and age of onset characteristic of polyglutamine disorders,[15] the behavioral phenotype became apparent at an earlier age in knock-in mice with 140 than 94 CAG repeats.[13] These early abnormalities, while highly reproducible, are mild, resembling the presymptomatic stages of the disease in which subtle motor anomalies occur.[16] Thus, knock-in models are useful tools to evaluate the ability of potential treatments to delay the onset of early abnormalities.

Similar to what is observed in patients suffering from HD,[2] knock-in mice with 140 CAG repeats display a reduction in the stride length at 12 months of age.[13] Gait abnormalities were also detected at 24 months of age in a separate line of knock-in mice with 109 CAG repeats.[17] Finally, another line of knock-in mice with 150 CAG repeats display a late-onset clasping, a progressive tendency to inactivity and gait abnormalities.[18] Differences in behavioral anomalies among knock-in mouse models, are not only due to the length of the CAG repeats they carry but also to differences in testing methodologies, differences in the genetic construct and/or the strain background. For example, the knock-in mice with 94 and 140 CAG repeats, in which early abnormalities were detected, were examined during the dark phase of the diurnal cycle, which corresponds to the period of highest activity in rodents.[13,14] Two different constructs were used to generate knock-in mouse models. In some models, the sequence codifying the polyglutamine stretch located in exon 1 of the *Hdh* mouse, homologous to the human *HD* gene, was replaced by a mutant polyglutamine repeat.[13,19,20] In others, the murine exon 1 was substituted by a quimeric *HD/Hdh* exon 1 with an expanded CAG repeat.[9,18] In both cases, the mutation is

expressed under the *Hdh* promoter in the full-length huntingtin protein. However, only the models that carry the chimeric *HD/Hdh* exon 1 carry a sequence encoding for the human polyproline tract that lies adjacent to the polyglutamine tract in huntingtin. Polyproline motifs are responsible for huntingtin interaction with SH3- and WW-domain-containing proteins, such as signal transduction and cytoskeletal proteins,[21–24] and they may be important to fully understand the interaction of mutant huntingtin with various proteins.[25] Furthermore, the polyproline region is likely to influence the folding of the protein. Finally, knock-in with 48, 90, and 109 CAG repeats are in Sv129 x CD1 background,[26] whereas knock-in with 71, 94, 140 are in Sv129 x C57Bl/6J background,[13,19] knock-in with 80 CAG and with 150 CAG repeats are in 129/Ola x C57Bl/6J,[18] and knock in with 72 and 80 CAG repeats are in Sv129 x C57Bl/6J and Sv129 x FVB/N.[9,27] These factors have to be taken into account when comparing the lines.

Knock-in mice are considered a precise genetic HD mouse model because they express the mutation in the murine huntingtin protein and the endogenous murine promoter controls its level of expression. Those characteristics could possibly explain the mild phenotype observed in the knock-in when compared to the more obvious phenotype observed in transgenic models. Some of these transgenic models express the mutation in a truncated protein,[11] which have been shown in *in vitro* studies to be more toxic than full length.[28,29] Others express the mutation in the full-length *HD* gene driven by the CMV promoter hence, achieving high levels of expression.

Whereas there are differences in the magnitude of motor abnormalities in knock-in and transgenic mice a shift from hyper- to hypoactivity was also observed in transgenic mice. For example, the R6/2 displayed hyperactive behavior at 3 wk of age but became hypoactive as they aged.[30] A similar pattern was observed in transgenic mice carrying the full-length *HD* gene with 48 and 89 CAG repeats.[31] Furthermore, deficits in knock-in were observed as early as 1 month of age in 140 CAG repeats knock-in,[13] which is around the time when abnormalities were observed in R6/2 mice[30,32] and much earlier than abnormalities were detected in YAC128 animals.[33] All together, this indicates that the abnormal behavioral phenotype observed in knock-in mice could be used as an early noninvasive outcome measure in the testing of new therapies.

CELLULAR, MOLECULAR, AND NEUROPATHOLOGICAL ABNORMALITIES

The neuropathological hallmark of HD is the selective loss of striatal medium spiny neurons and the development of astrogliosis.[1] Other regions, including cerebral cortex, globus pallidus, subthalamic nucleus, thalamus, hippocampus, and cerebellum show varying degrees of atrophy depending on the pathological grade.[1] Although none of the knock-in mice developed neuronal loss,[4] reactive gliosis was observed in the striatum of 14-month-old knock-in with 150 CAGs and, with incomplete penetrance, in 24-month-old knock-in with 109 CAGs.[17,18] Interestingly, axonal degeneration was observed in knock-in animals with 72–80 and with 150 CAG repeats at 17–22 and 14 months of age, respectively.[34,35]

Aggregates of mutant huntingtin have been detected both in postmortem tissue from patients affected by the disease and in numerous mouse models of HD.[4,36] Whether these aggregates are pathogenic, protective or incidental remains unclear. Nuclear staining and aggregates of the mutant huntingtin were observed in various knock-in models.[13,14,18,20,35] The early and slow progression of the neuropathology in knock-in mice permits a detailed regional and temporal analysis. Similar to what is observed in other polyglutamine disorders, an increase in the number of CAG repeats leads to a decrease in the regional selectivity of the neuropathology.[13,14,17,18,20,35] The first anomaly observed is nuclear staining, followed by microaggregates, and finally, nuclear inclusions. Nuclear staining and aggregates were restricted to the striatum in knock-in mice with 94 CAG repeats. These were preferentially located in the striosomal compartment of the striatum,[14] a region affected in the early stages of the disease.[37] In contrast, the pathology observed was more widespread in models carrying longer CAG repeats (109,[20] 140,[13] and 150 CAG repeats[18] mouse models). In knock-in mice with 140 CAG repeats, nuclear aggregates and staining of mutant huntingtin were detected as early as 1 month of age, first in the striatum and olfactory tubercle.[13] As the animals aged, they were also observed in the olfactory bulb, the nucleus accumbens, cerebral cortex, piriform cotex, hippocampus, and cerebellum.[13] Both in knock-in mice with 94 and 140 CAG repeats, aggregates of the mutant protein were consistently detected several months after the development of the behavioral anomalies,[13,14] supporting a growing body of evidence that suggests that aggregates are not solely responsible for the disease phenotype.[38,39] Furthermore, recent data suggest that aggregate formation reduces the amount of diffuse mutant huntingtin and prolongs cell survival.[40] Even though nuclear aggregates were observed in numerous regions in knock-in with 140 CAG repeats, they remained restricted to certain brain areas and were absent in the external and internal globus pallidus and substantia nigra pars reticulata,[13] for example, consistent with observations in humans.[41] Interestingly, the regions with early pathology all receive dense dopaminergic inputs, supporting a role for dopamine in HD pathogenesis.[42] Alternatively the regional selectivity of aggregate formation could result from impaired clear-

ance of mutant huntingtin[43] or differences in proteolysis.[44,45] Indeed, proteolitic cleavage of huntingtin may play a critical role in the disease. In knock-in mice with 150 CAG repeats increases in calpain cleavage products and calpain expression were detected.[45] Knock-in mice, by expressing the mutation in the full-length protein, are ideal to identify proteases and evaluate potential treatment targeting protein cleavage. Another potential mechanism that may relate to the cell-specific neuropathology observed is the somatic instability of the CAG repeat mutation which has been detected in the striatum of knock-in mice.[26,46] Parallel examination of 72-80 and 150 CAG repeats mice indicated that the initial size of the mutation influences both the onset and tissue-specific pattern of age-dependent, expansion-biased mutation length variability.[47] Interestingly, the absence of the mismatch repair gene Msh2 was sufficient to eliminate the striatal expansion-biased mutation variability in knock-in mice and to delay the onset of aggregate formation.[48]

In addition to nuclear aggregates, neuropil aggregates were also observed in various knock-in mouse models.[13,18,20,49] Whereas both types of aggregates were observed in numerous brain regions such as striatum and cerebral cortex, neuropil aggregates were also present in the output regions of the striatum, where nuclear aggregates were not observed.[13,35] This suggests that neuropil aggregates are located in the axons of striatal projection neurons. Neuropil aggregates appeared earlier in the external globus pallidus than in the internal globus pallidus. This is in agreement with evidence in brains of affected patients, which indicated that the striatal-external pallidal projections are affected earlier than the striatal-internal pallidal projections.[50–52] Whereas the role of neuropil aggregates in HD is unclear as well, data from a drosophila model of HD suggest that neuropil aggregates may have a deleterious effect by physically blocking axonal transport or synaptic transmission.[53] Indeed, neuropil aggregates were associated with late axonal degeneration in knock-in mice.[35] However, a deficit in synaptic transmission was detected before the formation of detectable aggregates.[27] Huntingtin may play a role in axonal transport through the interaction with HAP1,[54] which strongly associates with p150Glued, a critical component of the transport system.[55,56] Furthermore, disruption of axonal transport has been observed in the absence of aggregates.[57]

The striatal neuropathology observed in knock-in mice with 109 CAG repeats varied when examined in different strain backgrounds. Sv129 knock-in mice presented reduced levels of somatic instability and a delayed neuropathology compared to C57BL/6 knock-in mice, indicating the presence of genes that modify somatic instability in Sv129 strain background.[58] Hence, differences across the various knock-in models could be due to the strain in which the mutation is expressed.

The lack of overt cell death in mouse models of HD suggests that a long phase of cellular dysfunction is responsible for the abnormalities observed.[59] Transcriptional dysregulation could play an important role in HD. Sequestration of transcription factors by huntingtin aggregates is responsible for gene expression deficits.[59] Interestingly, however, in knock-in mice gene dysregulation was observed in the absence of aggregates.[60,61] Indeed, a decreased level of enkephalin mRNA was observed in 4-month-old 94-CAG-repeat knock-in mice several months before aggregates were detected by light microscopy.[60] Furthermore, recent evidence indicates that the soluble forms of mutant huntingtin interacts with transcription factors after undergoing conformational rearrangements.[62]

The cascade of events initiated by the mutation responsible for the striking selective vulnerability of the striatum in HD remains unknown. A recent study showed that, in transgenic mice, both the striatal neurons selectively affected and the striatal interneurons, which are not affected by the disease, displayed transcriptional alterations,[63] indicating that abnormalities in transcription do not account for the selective vulnerability observed in HD. Interestingly, the distribution of the earliest pathology in knock-in with 140 CAG repeats parallels that of dopaminergic innervation, suggesting that regions rich in this neurotransmitter that is known to produce oxidative stress, may be particularly prone to huntingtin misfolding. Recent evidence suggests that deficits in brain-derived-neurotrophic-factor (BDNF) supply could play an important role in the pathogenesis. BDNF is needed for the survival of striatal medium sized spiny neurons. It is produced by the cortex and delivered via the corticostriatal afferent. Both the loss of the beneficial function of normal huntingtin on BDNF expression and BDNF vesicle axonal transport could be involved in the selective vulnerability observed in HD.[64,65] Similar to what was observed in patients,[64,66] decreased levels of BDNF were observed in knock-in with 109 CAGs.[67] The decreased levels of cAMP and phospho-CREB could be responsible for the BDNF deficits,[67] supporting the hypothesis that energy impairment plays an important role in HD.

CONCLUSION

As a model for HD, knock-in mice have the advantage of carrying the mutation responsible for the disease in the appropriate protein context, the full-length protein, under the endogenous *Hdh* promoter. Some of these models develop behavioral, molecular, cellular, and neuropathological abnormalities at an early age. These effects can be used as biomarkers in the assessment of potential therapies. HD could be the consequence of both a gain of a toxic function of the mutant huntingtin and/or a loss of a beneficial function. This makes knock-in models ex-

cellent models for the assessment of proteases inhibitors, therapies that will modulate the toxicity of mutant huntingtin, and strategies that will restore the wild-type huntingtin function. The role of aggregates is still unclear. A large effort is devoted to the development of aggregates inhibitors. However, a growing body of evidence suggests that aggregates are not responsible for the early events triggered by the mutation.[40] Knock-in mice can be used to evaluate aggregate inhibitors, which might be beneficial if they inhibit aggregation by blocking the interaction of mutant huntingtin with critical cellular target or the formation of toxic mutant huntingtin. Because of the slow progression of the abnormalities detected, knock-in mouse models offer a unique possibility of dissecting the pathogenic mechanism of HD, in the precise genetic model of the human condition. The understanding of the pathogenic mechanisms will lead to the identification of new targets for therapeutic intervention.

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